THE CHROMOSOMES OF THE CERCOPIDÆ.

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The chromosomes of at least four species of this family have been already studied, one by Dr. N. M. Stevens,[1] and three by the writer.[2] These three species which I have already studied were collected at Cold Spring Harbor in 1907 and identified by Mr. E. P. Van Duzee as *Clastoptera obtusa*, *Aphrophora quadrangularis*, and *Aphrophora quadrinotata*. The species studied by Dr. Stevens was collected at South Harpswell in 1906 and identified at the time as *Aphrophora quadrangularis*, but the cytological differences from the Cold Spring Harbor material which was surely *Aphrophora quadrangularis* made Dr. Stevens later question the identification of the Harpswell material. The reduced number of chromosomes is different in all of these species, 12 in the Harpswell form, 15 in *Clastoptera obtusa*, 11 (12?) in *Aphrophora quadrangularis*, and 14 in *Aphrophora quadrinotata*. In other respects, the spermatogenesis in one species resembles that of the others, except for the Harpswell form which differs in several points: the odd chromosome of the early growth stages of the primary spermatocytes is long and rodlike; in later stages two M-chromosomes appear; and the odd chromosome in the equatorial plates of the primary spermatocytes is one of the medium-sized chromosomes. In the other species, the odd chromosome in the growth stages is never rodlike, but always rounded, and no M-chromosomes ever appear in the growth or division stages; the odd chromosome in the equatorial plates of the first spermatocyte division and in the sideview of the spindles appears to be one of the smaller chromosomes. In 1909, I collected material of the common European spittle insect, *Aphrophora spumaria*, at Eisenach, for chromosome study, and this summer at Woods Hole a chance to study one more species of this family was

[1] Stevens, N. M., 1906, "Studies in Spermatogenesis," Part II., Carnegie Inst., Washington.

[2] Boring, A. M., 1907, "A Study of the Spermatogenesis in Twenty-two Species of the Membracidæ," etc., *Jour. Exp. Zoöl.*, IV., p. 469.

afforded me by the abundance of *Philænus spumarius* on the goldenrod and wild sunflower. The results of the study of these last two species are given in this paper.

Material and Methods.

The *Philænus* material used in this study was found on the goldenrod and wild sunflower in the Fay woods at Woods Hole early in September. The adults were emerging rapidly from the nymph stage and the variation in marking was so striking that it seemed possible that the insects might belong to more than one species. However, specimens showing this variation were sent to Mr. E. P. Van Duzee, and he identified them all as *Philænus spumarius*. The same variations occurred among the insects on both goldenrod and wild sunflower. Nymphs from the two plants were kept separate in the laboratory until the adults appeared.

The *Aphrophora spumaria* material was collected from the grasses of a low meadow near Eisenach in September. All were adults at that time. This species exhibited as great somatic variations as *Philænus spumarius*, both in wing markings and the color of the abdomen.

The *Philænus* material used was partly from adults and partly from nymphs. The position and shape of the testes was exactly like that described for the material of the other species of Cercopidæ. In the just-emerged adults the testes show all stages of spermatogenesis, including fully formed spermatozoa, but in the females the ovaries are only slightly developed and difficult to find. There are only oögonia and very young growth stages of the primary oöcytes present. Evidently the eggs are not laid until the next spring.

For preserved material, the few posterior segments of the abdomen containing the testes were cut out and fixed in Gilson or in Flemming. But the most of the study of *Philænus* was made from acetocarmine preparations, and all the figures are drawn from such. This greatly expedited the study of the chromosomes in different individuals in connection with somatic variation. *Aphrophora spumaria* was studied entirely from preserved material.

Observations on Chromosome Individuality.

The chromosomes of *Philænus* are more like those of the three forms studied at Cold Spring Harbor than like the one worked on by Dr. Stevens at Harpswell. The abundance of the material and the large size of the chromosomes in the aceto-carmine preparations have made it possible to study the individuals in this form more accurately than previously in the other forms.

The equatorial plates of the primary spermatocytes always show 12 chromosomes (Figs. 1–7) and the secondary spermatocytes 11 and 12 (Figs. 8 and 9). The odd chromosome usually stands to one side of the primary spermatocyte plate, when it is in the same plane (Figs. 1–7, *X*), but it very often lies in an entirely different focus, at the end of the spindle (Figs. 10, 11, 12). It is always one of the smallest chromosomes. It is impossible to individualize each of the twelve chromosomes, but each plate shows one largest (*A*), two almost as large (*B*, *C*), several intermediate, and three or four smallest, one of which is the odd chromosome. The secondary spermatocyte plates show the same condition, one largest chromosome and two almost as large, but here in the plates with 11 chromosomes (Fig. 9), there is, of course, no odd chromosome and in the plates with 12 (Fig. 8), it has no particular position by which it can be identified.

The individuality of these chromosomes can be traced also in the prophase of the first spermatocyte division (Fig. 15). The odd chromosome is a single round body, while the others are already split for the first spermatocyte division. Among these dumbbell-shaped bodies the one largest and the two chromosomes almost as large are clearly recognizable (Fig. 15, *A*, *B*, *C*). In the side view of the metaphase figure the odd chromosome still appears round and undivided alongside of the other dividing chromosomes (Fig. 16). It is here about the size of half of one of the smallest bivalent chromosomes, so we could expect it to be undifferentiated by size from the smallest ones in the end view of the equatorial plates, as has been previously described.

In anaphase, the odd chromosome lags behind the others, but goes undivided to one pole. Its small size is again evident here (Fig. 17). The second spermatocyte division follows close after

the first. Fig. 18 shows the two chromosome groups from the ends of a spindle. Each chromosome is splitting, preparatory to the second division, as is also the odd chromosome. The two groups were not in the same focus, and it was clear that the odd chromosome belonged to the lower group. The size relations here are as before, one largest chromosome (*A*) in each group, and two almost as large (*B*, *C*). The odd chromosome (*X*) here appears slightly larger than the smallest, but they are rearranging themselves in shape and position for the second division, and therefore we cannot be sure that we are focusing on similar sides.

The equatorial plates in the spermatogonia and in the follicle cells are interesting to compare with those in the spermatocytes, for in both of the former, the unreduced number of chromosomes is present. The grouping in the spermatogonia is more compact than in the somatic cells (Figs. 19, 20), but in both there are two longest chromosomes (*AA*) and four almost as long (*BB*, *CC*), the three pairs represented by the one largest and two almost as large of the spermatocytes. In both also there is an odd number of chromosomes, 23, the condition invariably found in the spermatogenesis of forms with an odd chromosome. Which of the smaller chromosomes here is the odd one, there is no way to detect. In one somatic cell where the chromosomes are much longer and thinner than in any others (Fig. 22), the size relations are not so clear: there are two distinctly longest (*AA*), but apparently six next longest. However, the chromosomes are so long and twisted in this case that there is every possibility of having misrepresented their lengths in a flat drawing.

The equatorial plates of the oögonia and female somatic cells are similar to the corresponding male cells, except for the possession of one more chromosome, 24 instead of 23 (Figs. 23–25). This additional chromosome must be a small one, for there are only two longest (*AA*) chromosomes and four almost as long (*BB*, *CC*), as in the male cells. Presumably then this extra chromosome is the mate of the small odd chromosome of the male. Therefore, *Philænus spumarius* has dimorphic spermatozoa, and one more chromosome in all female cells than in the male cells, one usual expression of sex differentiation in insects.

Aphrophora spumaria has one chromosome distinctly larger than the others in the equatorial plates (Figs. 26–29). Sometimes there appear to be two intermediate-sized as in *Philænus*, but the cells and chromosomes are smaller and it is consequently not possible to be sure of small differences. The reduced number of chromosomes is 12, and one of these is an odd chromosome, which behaves in the typical way in division, like *Philænus* (Fig. 33, *X*).

The Odd Chromosome in the Growth Stages.

Similar as these two species are in the number of chromosomes, and the size relations of the chromosomes, it is striking to find the odd chromosome different in the growth stages. In *Philænus*, it is sometimes slightly oval in the earliest stages (Fig. 13), but more often round, and always round in the succeeding stages (Fig. 14, *X*). This is the condition in *Clastoptera*, *Aphrophora quadrangularis*, and *Aphrophora quadrinotata*. On the other hand, *Aphrophora spumaria* has a very long narrow dark-staining odd chromosome in the early growth stages (Fig. 30), which becomes round only in the stages just before the prophase of the first spermatocyte division (Figs. 31 and 32). Just such an odd chromosome was described by Dr. Stevens for the Harpswell form. But in *Aphrophora spumaria* there are no M-chromosomes. So there is variation in the Cercopidæ as to the shape of the odd chromosome in the growth stages.

Chromosomes and Somatic Variation.

The amount of variation in markings in *Philænus spumarius* made me suspect that they might be of more than one species. Some had distinct brown lines on the wings, forming a diamond, while others were of a light tan with only a dark streak at the posterior border. As one of the important cytological problems of the present day is to connect chromosomes with somatic variations, I made a careful study of the equatorial plates of different individuals with different markings. The plates of the first spermatocytes of seven differing individuals are shown in Figs. 1–7. There is evidently no clue to somatic variation here. The plates are strikingly alike, each has one largest chromosome

(*A*), and two almost as large (*B*, *C*), and most of them show a small odd chromosome (*X*) slightly to one side in the group. In Figs. 5 and 7, it is difficult to be certain which is the odd chromosome. Two somatic cells from two of these same individuals show also the same similarity in chromosomes, two longest and four almost as long (Figs. 20 and 21, *AA*, *BB*, *CC*).

The same sort of study in *Aphrophora spumaria* gave the same negative results. The somatic variation is great, but the chromosomes give no key to it. Fig. 26 is a plate from an individual with light spotted wings, Fig. 27 from one with light tan wings with no trace of spots, Fig. 28 one with dark spotted wings and black abdomen, and Fig. 29 one with streaked wings with the abdomen black on the sternites and yellow on the pleurites. All have one largest chromosome (*A*). There are probably two next largest as in *Philænus*, but that is no distinction between insects with different somatic characters. The only chance for chromosome variation among individuals lies in the peculiar small bodies shown in Figs. 34–37. These do not always stain as deeply as the chromosomes. They resemble somewhat the supernumeraries described by Stevens[1] in *Diabrotica*, and Wilson[2] in *Metapodius*, but they do not here occur condensed in the growth stages. There may be one, two, three, or four (Figs. 34–36). Whether this number is constant for each individual, I could not tell, as the testes from several individuals with similar wings and abdomen were preserved together. My material is so limited for the study of this point that I place these few drawings here in the hope that some one where the material is available will take the trouble to collect it and work out this point more thoroughly.

Summary.

1. The chromosomes of *Philænus spumarius* and *Aphrophora spumaria* were studied and compared with those of the four other species of Cercopidæ previously worked out. All have an odd chromosome which does not divide in the first spermato-

[1] Stevens, N. M., 1908, "The Chromosomes of *Diabrotica vittata*, *Diabrotica soror*, and *Diabrotica 12-punctata*," *Jour. Exp. Zoöl.*, V., p. 453.

[2] Wilson, E. B., 1909, "Studies on Chromosomes," V., *Jour. Exp. Zoöl.*, VI., p. 147.

cyte division. The odd chromosome in the early growth stages of the spermatocytes varies in shape in the different species.

2. The individuality of certain chromosomes was traced in these two species of Cercopidæ. In both, the reduced number of chromosomes is 12. In *Philænus*, the identity of one large and two medium-sized chromosomes can be traced in the primary spermatocyte, secondary spermatocyte, spermatogonium, oögonium and male and female somatic cells. The odd chromosome is always one of the smallest. In *Aphrophora*, one largest chromosome always appears.

3. The somatic variation in both species is very marked, but there is no corresponding variation in the composition of the chromosome groups.

University of Maine, Orono,
November, 1912.

All figures were drawn with a camera lucida, 1/12 oil immersion and 12 compensating ocular, then reduced in the engraving to four fifths. The drawings of Plates I., II., and III. are all from acetocarmine preparations, but those of Plate IV. are from material fixed in Gilson and stained in iron hæmatoxylin.

Explanation of Letters.

A = the largest chromosome.

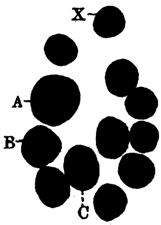
$\left.\begin{matrix}B\\C\end{matrix}\right\}$ = the two chromosomes almost as large as A.

X = the odd chromosome.

Explanation of Plate I. (*Philænus spumarius*).

Figs. 1–7. First spermatocyte metaphases from seven individuals with different somatic markings. All show 12 chromosomes, one largest (A) and two almost as large (B, C). Most of them show the odd chromosome at one side of the group (X).

Figs. 8–9. Metaphases of the secondary spermatocytes, one with 12 and one with 11 chromosomes.



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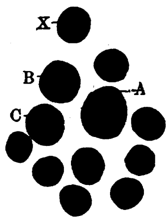

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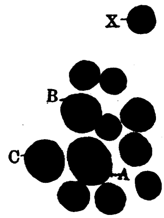

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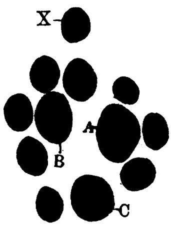

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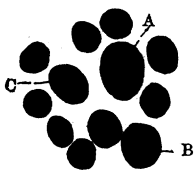

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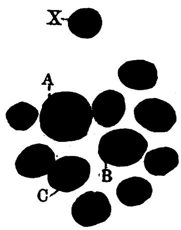

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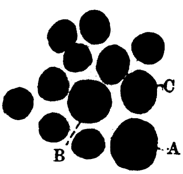

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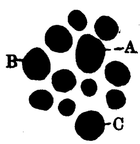

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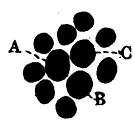

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EXPLANATION OF PLATE II. (*Philænus spumarius*).

FIGS. 10, 11. Two optical sections of one primary spermatocyte spindle such as is represented in Fig. 12, odd chromosome toward one pole.

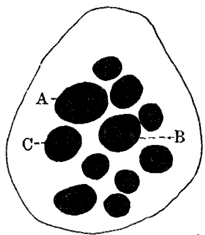
FIGS. 12, 16. Two metaphase spindles of the primary spermatocyte division.

FIGS. 13, 14. Early and late growth stages of the primary spermatocyte.

FIG. 15. Prophase of the primary spermatocyte, odd chromosome the only univalent one.

FIG. 17. Anaphase of the primary spermatocyte division, odd chromosome toward one pole.

FIG. 18. Two daughter plates of a primary spermatocyte division, each with 11 chromosomes, the odd chromosome between them.



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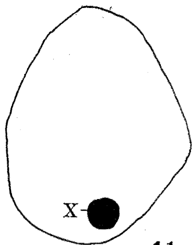

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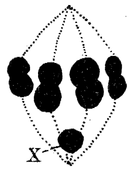

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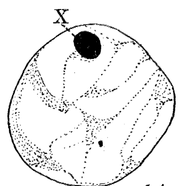

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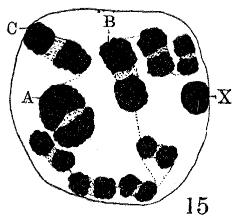

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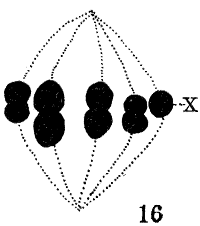

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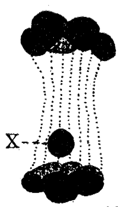

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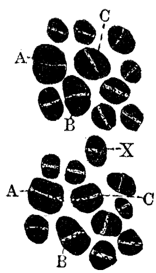

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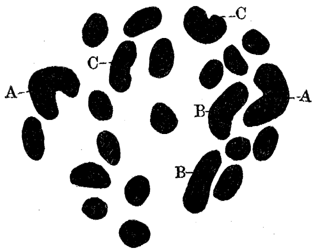
EXPLANATION OF PLATE III. (*Philænus spumarius*).

FIG. 19. Equatorial plate of a spermatogonium, 23 chromosomes, 2 largest (*AA*) and 4 next in size (*BB*, *CC*).

FIGS. 20–22. Male somatic cells, 23 chromosomes, size relations the same as in Fig. 19.

FIG. 23. Equatorial plate of an oögonium, 24 chromosomes, same size relations as in Fig. 19.

FIGS. 24, 25. Female somatic cells, 24 chromosomes, same size relations as in Fig. 19.



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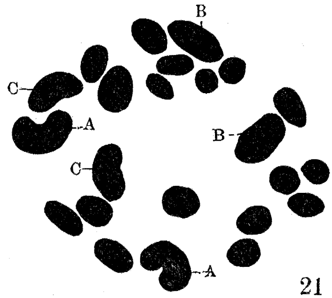

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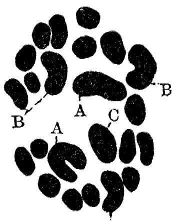

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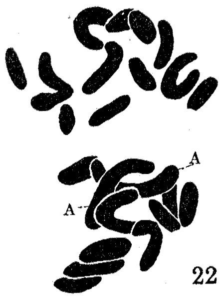

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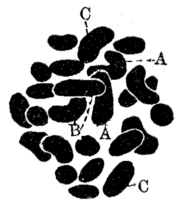

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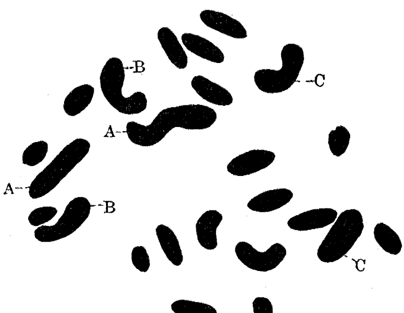

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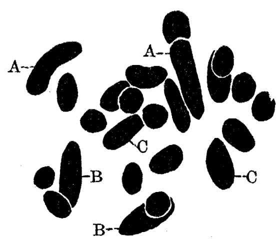

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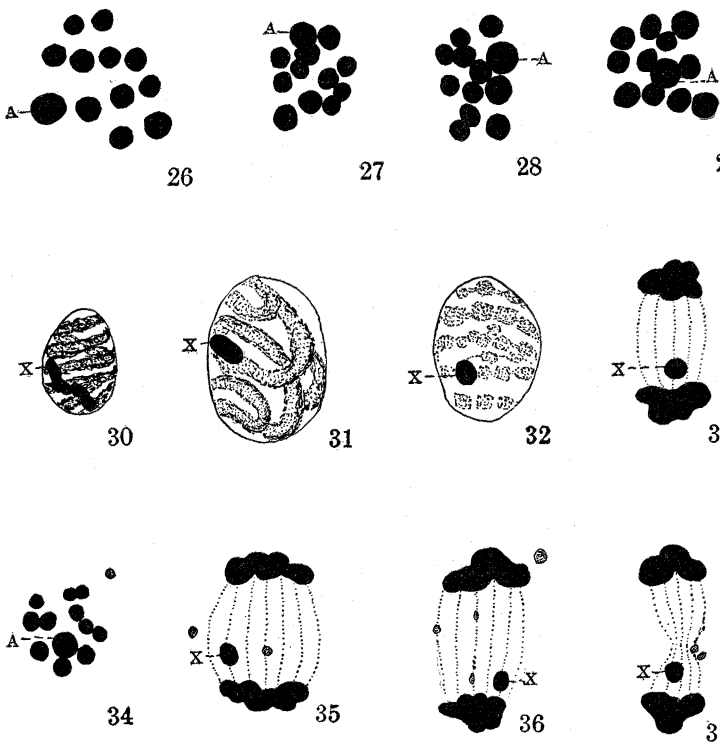
EXPLANATION OF PLATE IV. (*Aphrophora spumaria*).

FIGS. 26–29. Equatorial plates of primary spermatocytes of four different individuals with different somatic markings, one largest chromosome in each (A).

FIGS. 30–32. Growth stages of primary spermatocytes.

FIGS. 33, 35–37. Anaphases of primary spermatocytes, X toward one pole.

FIGS. 34–37. Drawings showing small chromatin bodies, which may be supernumeraries.



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